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ONLINE SUPPLEMENT

Short Title: Cardiovascular system in prior preeclamptic women

CARDIOVASCULAR SYSTEM DURING THE POST PARTUM STATE IN WOMEN WITH A HISTORY OF PREECLAMPSIA

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Additional Methodological Details

Systemic Arterial Hemodynamics and Mechanical Properties

With subjects in supine position, heart rate and blood pressure were measured in the non-dominant brachial artery using oscillometric sphygmomanometry (Critikon Dinamap, GE Healthcare, Wakesha, WI USA). Three separate blood pressure values were obtained and averaged. A custom Matlab® program (The Mathworks Inc., Natick, MA USA) was used to calculate hemodynamic variables from measured carotid pressure waveform (tonometry, see below), mean and diastolic brachial artery blood pressures (oscillometric sphygmomanometry), and cardiac output (echocardiography, see below) data. The carotid pressure waveform was assumed to be a surrogate for the aortic pressure waveform and was calibrated by equating mean and diastolic pressures at this site to those measured at the brachial site using oscillometric sphygmomanometry.[1] Total vascular resistance (TVR), calculated from the mean arterial pressure and cardiac output, was used to quantify the steady component of systemic arterial load. The pulsatile component systemic arterial load was characterized by global arterial compliance (AC_G). Two methods were used to estimate AC_G: analysis of the diastolic decay of the carotid pressure waveform using the area method[2] and as the ratio of stroke volume and carotid pulse pressure.[3]

Pulse Wave Velocity (PWV)

All measurements were made with subjects in supine position. ECG leads were placed on the subject and three vascular segment lengths were measured (heart-to-carotid, heart-to-femoral, and heart-to-brachial). A tonometer (Sphygmocor, AtCor Medical, Itasca, IL USA) was used to obtain pressure waveforms at three vascular sites (carotid, femoral, and brachial). Using the Sphygmocor machine, ECG and pressure waveform data were simultaneously recorded for 30 seconds (100 Hz sampling rate) at each of the three sites. These data were analyzed off-line by a custom Matlab® program. Specifically, transit time for the pressure wave to travel from the heart to a given vascular site was calculated as the time difference between the peak of the QRS wave on ECG and the foot of the pressure waveform at that site. The foot of the pressure waveform was identified using the intersecting tangent algorithm.[4-5] Heart-to-vascular site PWV was calculated as the ratio of the vascular segment length and pulse transit time.

Echocardiographic Assessment

All echocardiographic measurements were performed with subjects in left lateral decubitus position (GE Vivid 7, GE Healthcare, Wakesha, WI USA). Specifically, the following data were recorded: left ventricular dimensions in parasternal short axis (2D-directed M-mode) and apical and four chamber long axis (2D) views, left ventricular outflow tract diameter (D_{LVOT}) in parasternal long axis view, and aortic blood velocity obtained by continuous and pulsed wave Doppler from the apical window.[6] D_{LVOT} was measured during systole at the base of the aortic valve leaflets and aortic annular cross-sectional area was calculated assuming a circular orifice of diameter D_{LVOT}. Stroke volume was calculated as the product of aortic velocity-time integral and aortic annular cross-sectional area. Cardiac output was determined as the product of stroke volume and heart rate. Left ventricular mass was calculated using images at end-diastole and the area-length method as recommended by the American Society of Echocardiography.[7] End-diastolic and end-systolic left ventricular wall thickness (septal

and posterior) and chamber diameter were measured from M-mode, short-axis images. Left ventricular fractional shortening was calculated as the difference between end-diastolic and end-systolic chamber diameters divided by end-diastolic chamber diameter.

Endothelial Function

Forearm blood flow (FBF) was measured in the non-dominant arm by venous occlusion plethysmography (D. E. Hokanson, Bellevue, WA USA). A wrist cuff was inflated to supra-systolic pressure (200 mmHg) to exclude hand circulation while an upper arm cuff cycled between 15 seconds of inflation (50 mmHg) and 5 seconds of deflation during flow measurements. Baseline FBF was determined by the mean of six to eight FBF measurements. Subjects were then given a rest period of 15 minutes, allowing blood flow in the hand to return to normal. Next, the Stroop Color Word Test, a mental stress test that elicits an endothelial dependent vasodilation,[8] was administered over a period of three minutes. FBF was again measured under stressed conditions. Excess FBF was defined as the difference between stress FBF and baseline FBF and percent increase in FBF was calculated as excess FBF divided by baseline FBF. Systolic, diastolic, and mean brachial blood pressures, along with heart rate, were measured (Critikon Dinamap) in the dominant arm every 3 minutes during the rest period and every minute during the data acquisition period.

Blood Biochemical Analyses

Participants were asked to fast for at least eight hours prior to their study visit. Venous blood was collected using BD-vacutainer tubes and immediately centrifuged for 15 minutes at 4°C. Resulting plasma or serum samples were then separated and stored at -80°C in 1mL aliquots for later analysis. Testing of samples was segregated into four categories: (1) endothelial function [plasma cellular fibronectin (Millipore, Bedford, MA USA, elisa kit #08-102) and plasma E-selectin (R & D Systems, Minneapolis, MN USA, elisa kit #BBE-2B)], (2) dyslipidemia [serum triglycerides (Sigma, St. Louis, MO USA, diagnostic kit #343-25P), plasma apo B (Sigma, diagnostic kit #357-A), serum free fatty acids (Wako Chemicals, Richmond, VA USA, diagnostic kit #994-75409), plasma total Cholesterol and HDL (Sigma, diagnostic kit #402-20), and serum glycerol (Sigma, reagent #337-A)], (3) glucose homeostasis [plasma insulin and glucose (Sigma, reagent #115-A)], and (4) oxidative stress [plasma malondialdehyde (liquid chromatography)]. Homeostasis model assessment (HOMA) index was calculated as the product of fasting glucose and insulin values divided by 22.5.[9]

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Table S1. Left Ventricular Properties

Variable	Prior Uncomplicated Pregnancy	Prior Preeclamptic Pregnancy	*P* Value
LV mass (g)	140.7 ± 6.9	142.8 ± 10.2	0.872
D_{LVOT} (cm)	1.92 ± 0.02	1.91 ± 0.04	0.427
D_{LV_ED} (cm)	4.45 ± 0.07	4.61 ± 0.11	0.230
D_{LV_ES} (cm)	3.08 ± 0.06	2.92 ± 0.08	0.159
h_{PW_ED} (cm)	0.81 ± 0.02	0.79 ± 0.03	0.575
h_{PW_ES} (cm)	1.34 ± 0.03	1.37 ± 0.07	0.657
h_{S_ED} (cm)	0.75 ± 0.02	0.76 ± 0.03	0.934
h_{S_ES} (cm)	1.16 ± 0.02	1.17 ± 0.04	0.866
Fractional Shortening (%)	47 ± 1.6	59 ± 1.2	0.486

LV indicates left ventricle; D_{LVOT}, left ventricular outflow tract diameter; D_{LV_ED}, D_{LV_ES}, left ventricular end-diastolic and end-systolic diameter, respectively; h_{PW_ED}, h_{PW_ES}, left ventricular end-diastolic and end-systolic posterior wall thickness, respectively; h_{S_ED}, h_{S_ES}, left ventricular end-diastolic and end-systolic septal wall thickness, respectively. Data are mean ± SEM. ***P<0.05***, prior preeclampsia versus prior uncomplicated pregnancy by univariate logistic regression.